Stigma Receptivity and Seed Set in Protogynous Buffelgrass

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ABSTRACT

Buffelgrass, *Pennisetum ciliare* (L.) Link syn = *Cenchrus ciliaris* L., is an important warm-season perennial forage grass that is widely grown throughout the arid tropics. It has perfect florets, and emasculation is thought to be required to produce controlled hybrids. This is a tedious, difficult undertaking because of the small floret size. The flowering behavior of buffelgrass is such that the stigmas are exserted from the floret prior to anthesis, which is referred to here as the protogynous interval. This investigation was conducted to determine the duration of the protogynous interval in 447 buffelgrass accessions and to ascertain stigma receptivity during the protogynous intervals. Protogynous intervals in a field nursery near College Station, TX, for all accessions ranged from 1 to 4 d. Six accessions with protogynous intervals ranging from 1 to 3 d were used to investigate stigma receptivity under both self- and cross-pollinated conditions in a greenhouse. Pollen germination and tube growth were observed with fluorescent microscopy at different time increments following pollination. Across all accessions, pollen germinated within 15 min of contacting the stigma, and pollen tubes grew to the micropyle within 2 to 6 h, depending on the accession and pollen source. Mean seed set ranged from 11 to 76% and from 22 to 80% among accessions following self- and cross-pollination, respectively. This investigation revealed that variation exists for protogynous interval within buffelgrass, and the stigmas are receptive when exserted from the floret and remain receptive throughout duration of the protogynous interval regardless of whether it occurs 3, 2, or 1 d prior to anthesis. These findings demonstrate that protogyny can be used to produced controlled hybrids in sexual buffelgrass without emasculation.

Buffelgrass is a warm-season, perennial bunch grass that is an important forage in many of the drier regions of the world, including parts of the southwestern USA. The grass reproduces primarily by apomixis, with the mechanism being apospory followed by pseudogamy (Fisher et al., 1954; Snyder et al., 1955). Most buffelgrass accessions are obligate apomicts (Fisher et al., 1954; Snyder et al., 1955); however, facultative apomicts that reproduce by sexual and apomictic means also have been reported (Bray, 1978; Sherwood et al., 1980). Even though apomixis is prevalent within the species, Bashaw (1962) reported a unique sexual plant that has been used in genetic studies. Pseudogamous apomicts, such as buffelgrass, require fertilization of the polar nuclei for endosperm development. Therefore, viable pollen is a necessity for seed set in apomictic buffelgrass. Pollen viability also is important for hybridization in apomicts because when a facultative apomict is used as the female parent, new genotypes are produced when the reduced egg cells in meiotically derived embryo sacs are fertilized. Another fertilization event in apomict plants occurs when the unreduced egg in an apomictic sac is fertilized by a reduced sperm nucleus. This phenomenon is known as the fertilization of an unreduced egg ($2n + n$) or B_{III} hybridization (Bashaw and Hignight, 1990). Fertilization of an unreduced egg provides a means for producing new genotypes by the incorporation of whole alien genomes while retaining the entire somatic chromosome complement of the apomictic female parent. Bashaw and Hignight (1990) demonstrated that $2n + n$ fertilization can be used to develop unique apomictic buffelgrass germplasm.

A range of chromosome numbers has been reported for buffelgrass with the most common number being $2n = 4x = 36$ (Fisher et al., 1954). The species apparently is a segmental allotetraploid because its chromosomes typically pair as one or two quadrivalents and 16 or 14 bivalents during diakinesis of meiosis I. However, it is not uncommon to find plants with $2n = 5x = 45$ and $6x = 54$ chromsomes as well as aneuploids of these three ploidy levels (Fisher et al., 1954; Bashaw and Hignight, 1990).

Like most warm-season perennial grasses, buffelgrass is predominately cross-pollinated. Some accessions exhibit a protogynous flowering behavior where the stigmas are extruded from the florets prior to anther exsertion (Fisher et al., 1954; Snyder et al., 1955). The period between stigma exsertion and initiation of anthesis is referred to here as the protogynous interval. Little is known about the duration of this protogynous interval or the receptivity of the exserted stigmas prior to anthesis in buffelgrass. There are conflicting reports in the literature regarding stigma receptivity in buffelgrass. Snyder et al. (1955) indicated that the stigmas were exserted and apparently were receptive as early as 36 to 48 h prior to anthesis; however, Bashaw and Funk (1987) reported that the stigmas began to emerge 2 d before anthesis but were not receptive until the day of anthesis.

In the past, successful hybridization in buffelgrass has been accomplished by hand emasculation or with a gametocide prior to pollination. Because of the small size of the florets, hand emasculation is a tedious, difficult process which often results in damaging the pistil in an emasculated floret. An experimental gametocide that prevents anther extrusion from the floret has been used to produce controlled crosses in buffelgrass (Bashaw and Hignight, 1990). Unfortunately, this gametocide is no longer available and hand emasculation is the only option for producing controlled hybrids. Since protogyny might be a means of producing hybrids in buffelgrass without emasculation, this investigation was undertaken to (i) quantify the variation of protogynous intervals, (ii)

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Table 1. Pollen germination and tube growth of six buffelgrass accessions when self-pollinated 0 to 3 d prior to anthesis in the greenhouse.

Accession	Day pollinated prior to anthesis	Time after pollination	Pistils observed	Pollen grains observed	Pollen germination	Tubes to:		
						Style	Ovary	Micropyle
		h	no.		%	no.		
PI 409367	1	1	10	359	54	0	0	0
		2	10	498	60	2	0	0
		3	10	401	47	11	0	0
		4	10	509	45	45	13	6
		5	10	425	49	61	21	20
	0	1	10	248	57	0	0	0
		2	10	469	57	1	0	0
		3	10	476	64	30	0	0
		4	10	547	57	24	16	3
		5	10	727	70	65	22	12
PI 409407	1	1	17	618	47	0	0	0
		2	13	436	49	2	0	0
		3	11	430	42	14	1	0
		4	11	430	42	8	2	0
		5	13	492	30	19	6	2
	0	1	10	722	68	0	0	0
		2	10	542	55	1	0	0
		3	10	471	62	22	0	0
		4	10	464	55	28	14	3
		5	10	243	55	63	20	11
S 12103	2	1	11	513	75	0	0	0
		2	10	475	69	12	0	0
		3	11	1132	81	41	13	2
		4	11	1366	86	77	35	10
		5	10	1338	83	75	32	15
	1	1	10	775	89	0	0	0
		2	10	1565	88	44	0	0
		3	10	1447	86	69	18	6
		4	10	1625	82	68	23	12
		5	9	1333	83	45	20	16
	0	1	10	1138	81	0	0	0
		2	10	1410	91	30	0	0
		3	9	1358	83	46	12	8
		4	10	1561	88	63	20	16
		5	10	1458	86	65	21	18
PI 409704	2	1	6	100	54	0	0	0
		2	10	172	62	10	0	0
		3	10	238	62	18	1	0
		4	10	442	71	20	3	0
		5	10	268	66	27	6	3
	1	1	9	185	63	0	0	0
		2	10	710	59	1	0	0
		3	10	505	62	13	2	0
		4	10	621	64	27	8	4
		5	10	535	66	25	9	5
	0	1	11	397	58	0	0	0
		2	10	691	51	13	0	0
		3	10	535	46	13	0	0
		4	12	664	47	24	6	0
		5	15	951	52	23	9	5
PI 295657	3	1	10	424	80	2	0	0
		2	10	593	82	31	2	0
		3	11	516	78	30	12	5
		4	12	630	74	47	19	9
		5	12	613	80	51	20	12
	2	1	10	239	88	0	0	0
		2	11	497	86	23	4	0
		3	12	563	76	37	13	5
		4	10	525	74	39	16	8
		5	10	511	82	43	17	10

Continued.

determine the receptivity of stigmas during the different protogynous intervals, and (iii) determine seed set under both self- and cross-pollinated conditions during the different intervals for both sexual and apomictic buffelgrass accessions.

MATERIALS AND METHODS

Plant Materials

Seed of 447 buffelgrass accessions in the National Plant Germplasm System (NPGS) were germinated and 10 seedlings

Table 1. Continued.

Accession	Day pollinated prior to anthesis	Time after pollination	Pistils observed	Pollen grains observed	Pollen germination	Tubes to:		
						Style	Ovary	Micropyle
		h	no.		%	no.		
	1	1	11	758	77	0	0	0
		2	11	751	77	32	1	0
		3	10	469	78	25	10	5
		4	10	525	74	39	16	8
		5	10	623	83	53	23	15
	0	1	10	1074	85	4	0	0
		2	11	2172	78	40	3	0
		3	11	1277	77	63	28	11
		4	10	1194	81	75	36	23
		5	10	1271	79	81	42	34
PI 315679	3	1	9	288	78	0	0	0
		2	10	639	85	0	0	0
		3	10	678	87	39	22	8
		4	10	752	89	56	33	12
		5	10	858	88	69	37	16
		6	10	658	86	54	28	10
	2	1	10	576	84	0	0	0
		2	9	1461	90	0	0	0
		3	10	1340	85	61	16	5
		4	11	1984	82	85	25	7
		5	10	1383	87	69	20	6
	1	1	9	967	88	0	0	0
		2	10	228	83	0	0	0
		3	10	1226	85	61	20	7
		4	10	2098	79	75	33	14
		5	10	1234	87	69	35	13
	0	1	10	986	86	0	0	0
		2	10	871	80	0	0	0
		3	10	1236	83	41	18	5
		4	10	1809	76	56	27	12
		5	10	1254	84	68	33	14

of each accession were transplanted into individual pots in a greenhouse. After the seedlings had produced from 3 to 5 tillers, five plants of each accession were transplanted into a field nursery on the Texas A&M University Research Farm near College Station, TX, in April 1996 and were used to determine the variation for protogynous interval in this population. The interval between stigma exsertion and anthesis was determined by monitoring the florets in the upper one-third of a minimum of five inflorescences within each accession. Stigma exsertion date was recorded on a tag that was attached to the culm supporting the inflorescence, and the florets on each tagged inflorescence were examined daily to determine when anthesis had occurred.

Six accessions (PI 295657, PI 315679, PI 409367, PI 409407, PI 409704, and S 12103) were further evaluated for stigma receptivity and seed set because of their different protogynous intervals. PI 409407 and PI 409367 had 1-d protogynous intervals (short), PI 409704 and S 12103 had 2-d protogynous intervals (intermediate), while PI 315679 and PI 295657 had 3-d protogynous intervals (long). Five accessions (PI 295657, PI 315679, PI 409367, PI 409407, and PI 409704) were obligate apomicts or highly apomictic, whereas S 12103 was sexual. All had $2n = 4x = 36$ chromosomes except for PI 409704, which is a pentaploid with 45 chromosomes. Clones of each accession were planted into pots and grown in a greenhouse (35°C/25°C day/night) under a 12-h photoperiod with 1000-W high intensity discharge lamps. The protogynous interval of these six accessions was checked and verified under greenhouse conditions.

Pollination Techniques

The plant materials used to determine stigma receptivity and pollen tube growth and seed set were grown in a greenhouse under the above mentioned conditions. Individual inflorescences of each accession were placed into glassine bags prior to stigma exsertion. At approximately 0800 h of the following morning, the bag was removed from each inflorescence and the florets of each involucre were examined to determine if stigma exsertion had occurred. Once stigma exsertion had initiated, the date was recorded and each stigma was examined with a 16× hand lens to determine if pollen was present. All involucres with contaminated stigmas were removed from the inflorescences as were those with florets

Table 2. Seed set in buffelgrass when self-pollinated 0 to 3 d prior to anthesis in the greenhouse.

Accession	Day pollinated prior to anthesis	Florets	Caryopses	Seed set	SE
		no.		%	
PI 409697	1	423	123	24.3	11.9
	0	316	144	46.0	12.2
PI 409407	1	380	28	11.5	11.5
	0	386	52	14.0	5.0
PI 409704	2	203	76	39.0	3.0
	1	278	91	35.5	7.5
	0	291	109	38.5	1.5
S 12103	2	377	151	39.7	0.8
	1	317	137	45.0	5.0
	0	353	137	40.0	7.0
PI 295657	3	1054	539	51.7	4.4
	2	388	234	60.7	1.3
	1	345	206	59.7	4.3
	0	373	138	39.7	4.8
PI 315679	3	221	157	69.0	4.4
	2	270	205	75.7	8.5
	1	213	120	50.5	2.5
	0	420	290	70.0	8.5

Table 3. Pollen germination and tube growth of six buffelgrass accessions when pollinated with birdwoodgrass 0 to 3 d prior to anthesis in the greenhouse.

Accession	Day pollinated prior to anthesis	Time after pollination	Pistils observed	Pollen grains observed	Pollen germination	Tubes to:		
						Style	Ovary	Micropyle
		h	no.		%	no.		
PI 409367	1	1	10	1144	84	6	0	0
		2	10	1209	87	48	0	0
		3	10	945	86	38	4	0
		4	10	1511	88	93	17	2
		5	10	522	82	59	13	10
	0	1	10	1056	83	0	0	0
		2	10	1120	85	24	0	0
		3	10	1914	85	148	14	5
		4	10	1872	87	90	15	8
		5	10	966	84	79	21	15
PI 409407	1	1	10	1139	84	6	0	0
		2	10	1218	87	0	0	0
		3	10	944	86	83	4	0
		4	10	387	91	62	4	0
		5	10	268	84	73	11	8
		6	10	601	69	88	39	17
	0	1	10	917	84	0	0	0
		2	10	870	74	32	0	0
		3	10	635	65	40	2	0
		4	10	823	82	68	14	4
		5	10	918	85	105	34	16
S 12103	2	1	7	205	92	89	0	0
		2	12	201	93	109	14	11
		3	11	601	94	34	8	6
		4	11	669	88	52	24	8
		6	10	325	90	67	40	26
	1	1	11	1686	91	38	0	0
		2	11	2150	86	90	26	4
		3	10	3199	92	100	32	8
		4	11	1927	84	120	46	17
	0	1	11	2825	91	110	0	0
		2	10	3209	89	106	30	6
		3	11	4432	90	138	68	19
		4	11	3350	91	132	70	27
PI 409704	2	1	11	593	93	25	0	0
		2	12	984	93	78	21	1
		3	11	1674	88	121	59	14
		4	10	1113	88	115	83	30
	1	1	11	198	80	23	0	0
		2	10	186	80	136	9	1
		3	10	228	77	161	25	7
		4	7	339	88	45	27	10
		5	10	381	82	74	46	21
		6	11	416	86	70	55	16
	0	1	11	1507	86	0	0	0
		2	10	1312	90	45	0	0
		3	10	2674	84	107	36	9
		4	12	3465	83	148	80	21
PI 295657	3	1	10	415	86	0	0	0
		2	10	553	89	9	0	0
		3	10	647	92	57	20	5
		4	10	521	96	89	15	8
		5	10	532	92	88	19	11
	2	1	10	92	93	76	0	0
		2	10	74	95	54	0	0
		3	10	134	77	84	14	6
		4	10	94	96	62	12	3
		6	10	110	84	68	11	8

Continued.

in which stigma exsertion had not occurred. To determine when the stigmas were receptive, stigmas of florets on different individual inflorescences were hand-pollinated on consecutive days following stigma exsertion. Some inflorescences were pollinated the morning of stigma exsertion and the remaining inflorescences were enclosed in glassine bags. The following morning the bags were removed from some of the remaining inflorescences and the stigmas of florets on these inflorescences were pollinated. This was continued each morning until the day of anthesis at which time the remaining inflorescences with exserted unpollinated stigmas were pollinated prior to anther dehiscence. This protocol was followed for each accession and depending on the protogynous interval of the accession, pollinations were made on (i) 3, 2, 1, and 0 d for those with the long interval, (ii) 2, 1, and 0 d for those with the medium interval, and (iii) 1 and 0 d for those with the short

Table 3. Continued.

Accession	Day pollinated prior to anthesis	Time after pollination	Pistils observed	Pollen grains observed	Pollen germination	Tubes to:		
						Style	Ovary	Micropyle
		h	no.		%	no.		
	1	1	10	126	89	74	0	0
		2	10	208	90	120	0	0
		3	10	344	94	154	24	6
		4	10	132	85	88	18	8
		6	10	92	100	54	34	20
	0	1	10	1450	91	0	0	0
		2	10	1760	92	15	0	0
		3	11	1617	94	61	0	0
		4	11	2103	93	114	16	5
PI 315679	3	1	9	282	83	0	0	0
		2	10	251	65	6	0	0
		3	10	390	75	28	2	0
		4	10	452	89	55	22	4
		5	10	344	78	56	27	8
	2	1	10	128	83	80	0	0
		2	10	258	83	130	10	2
		4	10	202	88	108	34	24
		6	10	190	78	64	26	8
	1	1	10	222	89	30	0	0
		2	10	154	82	40	0	0
		4	10	418	83	104	44	26
		6	10	352	81	108	42	22
	0	1	11	1359	92	1	0	0
		2	10	2356	92	61	0	0
		3	11	2385	92	130	53	22
		4	11	3471	92	133	62	25
		5	10	2393	90	134	62	26

interval. After each inflorescence was pollinated, it was enclosed again in a glassine bag. This procedure was followed for those inflorescences used to determine stigma receptivity and pollen tube growth under both self- and cross-pollinated conditions, as well as those used to measure seed set for self- and cross-pollinated conditions.

Stigma Receptivity and Pollen Tube Growth

To determine stigma receptivity and pollen viability, 10 involucres were removed from selected inflorescences of each accession at different time intervals following pollination, fixed in FAA for 30 min., and stored in 70% (v/v) ethanol. Involucres were collected 15 min after pollination and thereafter at hourly intervals up to 6 h. Pistils were dissected from the primary florets and prepared for examination with fluorescent microscopy by a modification of Kho and Baër's (1968) technique. Pistils were placed in 1 *M* NaOH for 15 min, transferred into a 0.1% (w/v) aniline blue solution for at least 30 min, and examined with a Zeiss[1] Standard 14 microscope equipped with a F1 Epi-fluorescence condenser illuminated with an Osram HBO 50W high pressure Hg lamp (Carl Zeiss, Inc., Thornwood, NY). Pollen germination was determined by counting the number of germinated and non-germinated pollen grains on each stigma. Pollen tube growth was recorded as the maximum distance the tubes had grown into each pistil.

Stigma receptivity was determined for both self- and cross-pollinated conditions. For self-pollinations, the stigmas were dusted with pollen collected from plants of the same accession that were growing in a different greenhouse. Birdwoodgrass, *P. ciliare* (L.) Link var. *setigerum* (Vahl.) Leek, (PI 193444) pollen was used for the cross-pollination studies.

[1] Mention of a trade mark or a proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Seed Set

Seed set also was determined for all accessions under both self- and cross-pollinated conditions. The same pollination protocol described above was used for seed set determinations. Once the respective inflorescences were cross- or self-pollinated, they were immediately bagged and harvested 28 d later. A minimum of 200 primary florets was removed from the involucres of each inflorescence, and these were counted and threshed to dislodge the caryopses. Percentage seed set was calculated by dividing the number of caryopses by the number of primary florets harvested and multiplying by 100.

All data were tested for normal distribution and then analyzed by SigmaStat version 2 (SPSS, 1997). Data were analyzed by paired *t*-tests or ANOVA as appropriate. Significant differences were determined at the 0.05 probability level.

RESULTS AND DISCUSSION

Protogynous Intervals

The protogynous intervals were checked in the field nursery during June and July, 1996. Plant growth and flowering during this period appeared normal as both temperature and rainfall were similar to the 30 yr means. Of 447 accessions examined, 68 (15%) exserted their stigmas 1 d prior to anthesis, 343 accessions (77%) exserted their stigmas 2 d before anthesis, and the remaining 36 (8%) exserted their stigmas 3 d prior to anthesis. These findings demonstrate variability for length of the protogynous interval within buffelgrass. This range could be an important criterion in selecting accessions to use in a hybridization program, especially if early stigma exsertion can be used as a mechanism to circumvent emasculation in making controlled crosses.

Six of the accessions were grown in a greenhouse

under a 12-h photoperiod the following winter and the protogynous interval of each accession was the same as when the plant was grown in the field the previous summer. This suggests that protogynous interval is a consistent trait that is not influenced by different environmental conditions.

Self-Pollination

Pollen Germination and Tube Growth

Pollen tubes were observed within 15 min of contacting the stigmas in all six accessions, regardless of the protogynous interval or the day within the protogynous interval when pollinations were made. Mean pollen germination under self-pollination ranged from 51 to 84% across all six accessions (Table 1). There were significant ($P < 0.05$) differences in pollen germination between the three protogynous interval groups. The two accessions (PI 409367 and PI 409407) selected for the short-interval group produced pollen of lower viability. The reason for their lower germination is unknown. Accession PI 409704 of the intermediate group also had a relatively low mean germination of 59% (Table 1). The most likely reason for the lower germination is that this accession is a meiotically irregular pentaploid with 45 chromosomes; the other accessions are stable tetraploids with 36 chromosomes. During meiosis, the 45 chromosomes associate essentially as 18 bivalents and 9 univalents. The univalents tend to lag behind the other chromosomes during anaphases I and II, and form micronuclei at telophases I and II which often are not incorporated into the nuclei. Consequently, the resultant pollen grains lack chromosomes and viability is reduced. Pollen germination for the remaining accessions (PI 285657, PI 315679, and S 12103) was 80% or higher (Table 1). Differences in pollen germination between these three accessions and the two selected for the short-interval group appear to be genotype specific. Thus, germination does not appear to be associated with the protogynous interval or influenced by the length of time the stigmas have been exserted from florets prior to pollination.

Following pollen germination, the tubes immediately elongated and penetrated the stigma papilla where they continued growing through the stigma branches and into the central axis of the stigma. With the exception of PI 315679, the time required for the tubes to grow into the style, regardless of when the stigmas were pollinated, was 2 h or less (Table 1). Pollen tube growth through the stigma in PI 315679 was slower than the other accessions; however, once the tubes entered the style, their growth into the ovary and to the micropyle was rapid because they were observed to be at the micropyle within 3 h (Table 1). For all six accessions, the pollen tubes reached the micropyle within 3 to 5 h following pollination. It appeared to take more time for the tube grow to the micropyle in PI 409704 than the other accessions. This slow growth rate could be associated with the high frequency of aneuploid pollen resulting from the meiotic irregularities in this pentaploid accession.

Pollen tube growth through the pistil under self-pollinated conditions appeared to be slower in buffelgrass than other warm-season grasses. In dallisgrass, *Paspalum dilatatum* Poir., and *P. juergensii* Hackel, the tubes grew to the micropyle within 45 min after pollination (Burson, 1987). The same was true for kleingrass, *Panicum coloratum* L.; however, in *P. deustum* Thunb. and *P. antidotale* Retz., from 1.75 to 2 h were required for the tubes to reach the micropyle (Burson and Young, 1983). An important finding from this study is that the pollen tubes were observed at the micropyle, whether pollination occurred 3, 2, 1, or 0 d prior to anthesis. These results support the conclusions of Snyder et al. (1955), who reported that buffelgrass stigmas were receptive up to 48 h prior to anthesis.

Seed Set

Seed set under self-pollination ranged from 11.5 to 75.7% (Table 2). Previous studies have reported self-pollinated seed set in buffelgrass to range from 2 to 84% (Read and Bashaw, 1969) and 27 to 91% (Hignight et al., 1991). Mean seed set for the short, intermediate, and long protogynous interval groups was 24, 40, and 60%, respectively (Table 2). Significant ($P < 0.05$) differences were observed between the three interval groups, as well as between the accessions within the short and long interval groups. Those accessions with lower pollen germination generally had lower seed set. Seed set was not influenced by what day during a protogynous interval pollination occurred. Seed set data demonstrate that the pollen tubes are proceeding through the micropyle into the female gametophyte and fertilization is occurring. This is most evident in the sexual accession S 12103 which requires double fertilization for seed development. It is also true for the five apomictic accessions because they are pseudogamous and are believed to require fertilization of the polar nuclei for endosperm development.

Cross-Pollination

Pollen Germination and Tube Growth

Pollen germination and tube growth were examined for each accession when pollinated with pollen from the birdwoodgrass accession PI 193444. Regardless of accession, protogynous interval, or the day within the protogynous intervals that the stigmas were pollinated, the pollen germinated within 15 min of contacting the stigmas. Percentage germination was much higher under cross-pollination than self-pollination, with the exception of PI 315679 in which they were about the same (Tables 1 and 3). With cross-pollination, mean germination ranged from 81 to 91% for the six accessions. The birdwood accession PI 193444 was used as the pollinator because it produced good quality pollen (Read and Bashaw, 1969). Findings from this study confirm their reports (Table 3).

Immediately following germination, the tubes began elongating and penetrated the stigma papillae. In most accessions, the tubes grew through the stigmas into the styles faster when cross-pollinated than when self-polli-

nated. This was evident for PI 409704, S 12103, and PI 315679 (Tables 1 and 3). Tube growth through the ovaries and to the micropyle in the sexual accession S 12103 and the pentaploid accession PI 409704 also occurred more rapidly than when self-pollinated (Tables 1 and 3). The reason for this behavior in PI 409704 is probably because of the difference in the quality of its aneuploid pollen and the euploid birdwoodgrass pollen. An exception to the faster pollen tube growth with cross-pollination was observed for PI 315679 (Tables 1 and 3).

For all accessions, regardless of which day within the three protogynous intervals that the stigmas were pollinated, birdwoodgrass tubes grew to the micropyle within 2 to 5 h following pollination and in most cases within 2 to 4 h (Table 3). Approximately the same amount of time was required for the tubes to grow to the micropyle in interspecific crosses among three *Paspalum* species; however, in two of the crosses, tubes reached the micropyle within only 30 to 45 min after pollination (Burson, 1987). Findings from our study demonstrate that stigmas of these six buffelgrass accessions are receptive to birdwoodgrass pollen when they are exserted from the floret and remain receptive until anthesis regardless of the protogynous interval.

Seed Set

When the six accessions were crossed with birdwoodgrass, seed set ranged from 22.0 to 80.4% (Table 4). Significant ($P < 0.05$) differences were observed among the protogynous interval groups with a mean seed set of 33, 50, and 69% for the short, intermediate, and long interval groups, respectively. Overall, cross-pollination resulted in 21% higher seed set than self-pollination, with the only exception being the sexual accession, S 12103. These findings are similar to those reported by Hignight et al. (1991) who reported that when buffelgrass was open-pollinated, 23% more seed were produced than when self-pollinated. This is consistent with the fact that buffelgrass evolved as an out-crossing species. Similar increases have been reported by pollinating *Bothriochloa* accessions with pollen from *Dicanthium* species (Dewald and Harlan, 1961).

For all six buffelgrass accessions, seed was produced when cross-pollinated 3, 2, 1, and 0 d prior to anthesis. For the apomictic accessions, this demonstrates that cross-pollination prior to anthesis results in pseudogamous seed development. For the sexual accession S 12103, these seed set data demonstrate that not only do the pollen tubes reach the micropyle, but continue through the micropyle into the embryo sac where the sperm nuclei present in the tubes fertilize the egg and polar cells.

This investigation revealed that buffelgrass germplasm varied in protogynous interval from 1 to 4 d in length. We also determined that buffelgrass stigmas are receptive to both buffelgrass and birdwoodgrass pollen when they are exserted from the floret, and they remain receptive until anthesis. These findings demonstrate that the protogynous nature of buffelgrass can be used to produce hybrids in sexual genotypes without having to make tedious, time-consuming hand emasculations. Seed set in apomictic genotypes that produce small quantities of seed might be increased if pollinated with other sources.

Table 4. Seed set in buffelgrass when pollinated with birdwoodgrass 0 to 3 d prior to anthesis in the greenhouse.

Accession	Day pollinated prior to anthesis	Florets	Caryopses	Seed set	SE
		——— no. ———		%	
PI 409367	1	435	177	34.0	9.9
	0	955	417	42.1	5.4
PI 409407	1	721	254	35.2	5.4
	0	235	50	22.0	7.0
PI 409704	2	314	175	55.5	2.5
	1	210	107	50.0	10.0
	0	408	255	62.5	0.5
S 12103	2	539	274	50.2	6.2
	1	309	136	44.5	15.5
	0	442	163	37.7	3.8
PI 295657	3	582	329	57.2	6.3
	2	513	340	64.0	4.4
	1	271	205	75.0	2.1
	0	235	129	55.0	1.0
PI 315679	3	426	341	79.0	3.6
	2	1330	956	72.0	4.6
	1	705	578	80.4	5.4
	0	269	183	68.0	3.4

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